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RESEARCH ARTICLE

Ultrasound with controlled temperature as an emerging technology for extraction of antioxidant compounds from by-products of mango (*Mangifera indica* L. *var* Ataulfo) juice

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ABSTRACT

During the industrial processing of juice high amounts of by-products are obtained which are discarded. The objective of this study was to optimize the conditions of ultrasound process using response surface methodology (RMS) based on antioxidant compounds extraction (total phenolic content (TPC) and ascorbic acid (AA) and antioxidant activity (ABTS and DPPH) of mango waste and its comparison with two conventional extraction methods (aqueous and hydroalcoholic). All response variables were fitted to the mathematical model ($R^2 \geq 95$). The optimal processing conditions corresponded to 91% of amplitude with a treatment time of 7 min, reaching the highest extraction of TPC and AA, as well as antioxidant activity. And in comparison, to conventional extraction methods (aqueous and hydroalcoholic), the optimal ultrasound conditions had ($p < 0.05$) highest antioxidant content with 8800 mg GAE/100 g dry weight (dw) to total phenolic content, 5600 mg AAE/100 g dw in ascorbic acid, 60000 and 7500 µmol TE/100 g dw for DPPH• and ABTS•$^+$ assays, respectively. These results demonstrate that ultrasound is an alternative method to enhance the extraction of antioxidants from mango industrial waste which could be used as additives in food products.

Keywords: Anti-radical activity, Mango (*Mangifera indica* L.), Response Surface Methodology, Tropical fruit.

INTRODUCTION

Mangifera indica L. (mango), "the king of fruits" belonging to the family *Anacardiaceae*, is one of the most popular fruits in tropical regions. Mango has been cultivated for 4000 years and ranks only second to pineapple in quantity and value among internationally-traded tropical fruits (Abbasi et al., 2015; Vithana et al., 2019; Lebaka et al., 2021). Mangoes are commercially cultivated in more than 100 countries worldwide and their demand is increasing in the international market, projecting their annual economic value to increase by 7.2% from 2019 to 2025 (Tirado-Kulieva et al., 2021). During the last decades, nine countries account for more than 80 % of total world production: India with 13.6 million tons is the leading producer followed by China, Indonesia, Mexico, Thailand, Pakistan, Brazil, and Nigeria (FAO, 2021). México is the leading supplier of mangoes globally, registered a 13.3-percent growth in 2020, to reach 465000 tons, an equivalent of 21 percent of total global traded quantities. On average, nearly 90 percent of Mexican mango exports are destined to the United States, with the remainder shipped to Canada (FAOSTAT). Besides, Mexico is the only producer and exporter of the Ataulfo mango variety of appellation of origin (Mendoza-Herrera et al., 2020). In the industrial processing of mango, by-products represent between 35–60 % of the fresh fruit weight, 15-18% provided by skin residues, and 13-29 % by seeds, which with pulp fraction comprises a considerable waste. In addition, a regular 3-month production season represent over 7,500 ton of polluting by waste (Guzmán et al., 2010; Sumaya-Martínez

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et al., 2012; García- Magaña et al., 2013; Lim et al., 2019). Several studies have evaluated the potential use of mango agro-industrial waste as a source of bioactive compounds (Ajila et al. 2007; Mejía et al., 2007; Castro-Vargas et al., 2019). By-products from Mango are rich in pectin with a significant galacturonic acid concentration, polyphenols as Mangiferin and polyunsaturated fatty acids that have antioxidant and anti-inflammatory properties (Ajila et al., 2007; Wongkaew et al., 2021, Lebaka et al., 2021), skin-protecting, neuron-protecting, antimicrobial, and prevention of cardiovascular diseases and cancer (Jahurul et al., 2015; Lebaka, et al., 2021; Lou et al., 2010). In addition, other compounds as dietary fiber with health-promoting potential include lowering blood cholesterol and sugar levels, improving cardiovascular health, as well as potential food additive or as a functional food ingredient, which can meet the techno-functional purposes required for developing health-promoting value added products (Hussain et al., 2020). For the above, emergent technologies such as ultrasound-assisted extraction (UAE) has been applied with finality to obtain bioactive compounds. The UAE is a simple, fast, and efficient technique compared to conventional extraction methods (Zhao, Y. et al. 2014). When high-power ultrasound is applied, liquid propagates and generates cavitation bubbles that imploded and collapse (Valdramidis et al., 2010), these forces disrupt cell structures allowing the penetration of solvent into the cell tissue and the release of their content, UAE is considered as a green technique, as it requires low solvent volumes for the extraction and constitutes an efficient technology in terms of energy consumption (Carreira-Casais et al., 2021). Besides, UAE also has been considered an efficient method to extract bioactive compounds from different materials by applying lower temperatures and shortening extraction time (Zhao et al., 2014; Ho et al., 2014., Altemimi et al., 2016). In addition, the use of response surface methodology (RSM) has been applied to optimize the parameters of ultrasound methodology (i.e. extraction temperature, power percentage, and exposure time) for bioactive compounds extraction. Thus, they are usually combined with finality to obtain a higher yield (Riciputi et al., 2018; Md Yusof et al., 2019; Morales et al., 2020). Therefore, the purpose of this study was to obtain the optimal ultrasound conditions using RSM to extract phenols and ascorbic acid and measure the antioxidant activity of the residues from the processing of Mangoes (*Mangifera indica* L.) *var.* Ataulfo, as well as a comparison of optimal ultrasound conditions with conventional extraction methods.

MATERIALS AND METHODS

Sample preparation

Mangoes (*Mangifera indica* L.) *var.* Ataulfo were collected at the community of San Blas, Nayarit, Mexico (geo-coordinates: N21° 32' W 105° 19', 1200 m above sea level). By-products (peels, pulp residues and seeds) were obtained after pulping 10 kg of mango using small-scale pilot plant equipment (pulper machine, Jersa 3009406, Mexico) located in the Autonomous University of Nayarit. The residue was passed through a spray machine and a hammer mill (Veyco MPV 400 Jumbo, Mexico) where a paste was obtained as waste. This waste was lyophilized (7753020, Labconco, USA), milled and sieved to a particle size of 500 μm, and stored in frozen at -30 °C until analysis.

Ultrasound treatment

The ultrasound extraction process was carried out according to Zafra-Rojas et al., (2016) using an equipment VCX-1500 (Sonics & Materials, Inc. Newtown, CT, USA) at 1500 W with a constant frequency of 20 kHz. The lyophilized residue was prepared at 4% solution (w/v) with deionized water. The solution was introduced in a jacketed vessel, (1210610, Col-Parmer, USA) to circulate water (4 ± 1 °C) in the secondary layer with the purpose of controlling the heat during the ultrasound process and the jacketed vessel was closed using a probe of 25 mm. The ultrasound conditions were amplitude levels of 80 - 90% and time of 5 - 15 min (pulse durations of 2 s and 4 s to on and off, respectively). The outlet temperature after ultrasound application was 25 ± 1 °C. Ultrasonicated samples were centrifuged at 15300*g* (Allegra 25™, Beckman Coulter, CA, USA) for 30 min at 4 °C and the supernatant was stored in frozen at -30 °C until analysis.

Experimental design

Response surface methodology was used to obtain the optimum ultrasound condition on the extraction of antioxidants from mango by-products. A central composite rotatable design using two independent variables at five levels was applied. The independent variables used were: amplitude (X_1, %) and sonication time (X_2, min), with range between 80-90% and 5-15 min, respectively. The design consisted in thirteen combinations with five central point replicates (Table 1). With values obtained for each response variables, a multiple nonlinear regression analysis was applied using the JMP® 5.1 statistical software (SAS Institute Inc., USA) to fit a second-order polynomial model:

$$Y_i = \beta_0 + \sum_{i=1}^{2} \beta_i X_i + \sum_{i=1}^{2} \beta_{ii} X_i^2 + \sum_i \sum_{j=i+1} \beta_{ij} X_i X_j$$

Where *Yi* is the predicted response; β_0 the constant coefficient, β_i the linear coefficient, β_{ii} the quadratic

Table 1: Ultrasonic conditions for mango waste treatment.

	Factor levels				
Independent variables	–α	–1	0	+1	+α
Amplitude (%)	78	80	85	90	92
Time (min)	3	5	10	15	17

coefficient, β_{ij} is a cross-product coefficient. X_i and X_j are independent variables. The adequacy of the mathematical model was determined using the coefficient R^2. By means of SigmaPlot 10.0 graphing software (SYSTAT Software Inc., Richmond, CA, USA), three-dimensional response surface plots as well as superposing contour plots were performed. To validate reproducibility, the predicted values given by the quadratic empirical model were compared with experimental values using t-test at significant level of $p<0.05$ by means of SPSS® System for WIN™ version 15.0.

Conventional extraction methods

Two conventional extraction methods (hydroalcoholic and aqueous) were performed to compare with optimized extraction by ultrasound process. For both methods (ethanol (80%) or deionized water), twenty grams of lyophilized sample were used and for extraction, an overhead stirred (HS-50A, Wisestir® Wisd, laboratory instruments, Korea) was performed 3 times: 60 min (160 mL), 30 min (80 mL) and 30 min (80 mL) (Vulic et al., 2011). The three extractions were centrifuged separately at 15300*g* during 30 min at 4 °C (Hettich, Mikro 22r, USA) and supernatants were combined and stored frozen at -30 °C until analysis.

Total phenolic content

Total phenolic content (TPC) was spectrophotometrically determined using the Folin-Ciocalteu method (Singleton et al., 1965). One-hundred microliters of sample solution were mixed with 500 µL of Folin-Ciocalteu reagent. Then, 400 µL of sodium carbonate was added and the mixture was incubated for 30 min at room temperature. The mixture absorbance was measured at 750 nm in a microplate reader (Power Wave XS UV-Biotek, software KC Junior, USA). Gallic acid was used as a standard, and results were expressed as milligrams of gallic acid equivalents per 100 g dry weight (mg GAE/100 g dw).

Ascorbic acid content

Ascorbic acid content (AA) was determined according to Dürüst et al. (1997). One-hundred microliters of sample solution were mixed with 100 µL of buffer (sodium acetate/distilled water/glacial acetic acid) and 800 µL of 2,6-Dichloroindophenol (DCIP). The mixture absorbance was measured at 520 nm in a microplate reader (Power Wave XS UV-Biotek, software KC Junior, USA). Oxalic acid was used as blank, and ascorbic acid was used as reference standard. Results were expressed as milligrams of ascorbic acid equivalents per 100 g dry weight (mg AAE/100 g dw).

Determination of antioxidant activity

Antiradical activity determination by DPPH•

Antiradical activity was measured with 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•) according to Morales and Jiménez-Pérez (2001). Five-hundred microliters of DPPH• solution (7.4 mg DPPH• in 100 mL ethanol) were added to a 100-µL aliquot of the extracts. The mixture was allowed to stand in the dark at room temperature for 1 h, and then vortex-homogenized. Absorbance was measured at 520 nm in a microplate reader (Power Wave XS UV-Biotek, software KC Junior, USA). A 0-300 µM series of Trolox solution in ethanol was used as reference. Antioxidant activity was expressed as micromol of Trolox equivalents per 100 g dry weight (µmol TE/100 g dw).

Antiradical activity determination by ABTS•$^+$

2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS•$^+$) antiradical capacity was measured according to Kuskoski et al., (2005). Briefly, the radical ABTS•$^+$ was produced by reacting a 7-µmol ABTS•$^+$ stock solution with a 2.45-µmol potassium persulfate solution in the dark at room temperature for 16 h before use. The ABTS•$^+$ solution was diluted with deionized water to an absorbance of 0.70 ± 0.10 at 754 nm. The sample was diluted in deionized water (1:5), and a 20-µL aliquot of diluted sample solution was added to 980 µL of the diluted ABTS•$^+$ solution. Absorbance was measured at 754 nm in a microplate reader (Power Wave XS UV-Biotek, software KC Junior, USA) after 7 min of incubation at room temperature. A 0-300 µM series of Trolox solution in ethanol was used as reference. Antioxidant activity was expressed as micromol of Trolox equivalents per 100 g dry weight (µmol TE/100 g dw).

Statistical analysis

All variables used to compare the optimized ultrasound extraction with conventional methods were obtained from three independent experiments; each sample was analyzed in triplicate (n = 9) and expressed as means ± standard deviation (SD). One-way ANOVA was used and differences between means were determined using a Tukey test, as well as a Student test was performed to validate reproducibility by comparing the predicted values given by the quadratic empirical model with experimental values with a level of significance of $p < 0.05$ using the IBM SPSS Statistics package, version 20.

RESULTS AND DISCUSSION

Total phenolic and ascorbic acid content

Table 2 shows the total phenolic content (TPC) in mango waste obtained after ultrasound extraction. Values ranged from 8137.68 to 9227.17 mg GAE/100 g dw, and according to regression coefficients (Table 3), all response variables presented high correlation with the mathematical model with values of $R^2 \geq 95$. The amplitude-time interaction (β_{12}) had a significant effect on TPC ($p < 0.0001$) as shown in

Table 2: Experimental design and TPC, AA and antioxidant activity in mango waste subjected to extraction by ultrasound[a]

Run	Amplitude (%)	Time (min)	TPC (mg GAE/100g dw)[b]	AA (mg AAE/100g dw)[c]	DPPH• (µmol TE/100g dw)[d]	ABTS (µmol TE/100g dw)
1	85	10	8854.64	5917.12	60321.00	69808.33
2	90	5	8622.46	5475.54	59902.78	72708.33
3	90	15	8902.17	6040.22	54416.67	86800.00
4	85	10	9016.85	5917.12	59044.44	69479.17
5	92	10	8902.25	5780.11	56967.59	81145.83
6	80	5	9227.17	5766.52	49825.00	78541.67
7	85	10	8850.72	5861.82	59166.67	71870.00
8	78	10	8493.48	5700.87	61259.26	87833.33
9	85	17	8549.64	5618.21	60504.63	76083.33
10	80	15	8137.68	5366.85	66365.00	82187.50
11	85	10	9015.22	6014.40	60876.00	72875.00
12	85	10	8997.10	6026.63	61293.00	69765.00
13	85	3	9044.20	5557.07	54875.00	65437.50

[a]Values are the mean±standard deviation (n=3), [b]milligram of Gallic Acid Equivalent per 100 grams dry weight, [c]milligram of Ascorbic Acid per 100 grams dry weight, [d]micromol of Trolox Equivalent per 100 grams dry weight

Table 3: Regression coefficients and ANOVA of regression parameters of the predicted response surface quadratic models for TPC, AA and antioxidant activity

Coefficient	TPC	AA	DPPH•	ABTS•+
β_0	8946.905[a]	5947.417[a]	60140.222[a]	70759.500[a]
β_1	92.233[d]	61.806[c]	-992.486[d]	-1334.798
β_2	-188.650[b]	31.433	2376.923[b]	4099.122[b]
β_{12}	342.300[a]	241.086[b]	-5506.528[a]	2611.458[d]
β_{11}	-130.776[c]	-103.909[c]	-706.963	7473.635[a]
β_{22}	-81.247[d]	-180.335[b]	-1418.769[d]	609.052
R^2	0.95	0.96	0.95	0.95

β_0 the constant coefficient, β_1 (amplitude), β_2 (time) the linear coefficient, β_{11} y β_{22} the quadratic coefficient, β_{12} is the cross-product coefficient. Levels of significance: a, $p < 0.0001$; b, $p < 0.001$; c, $p < 0.01$; d, $p < 0.05$

Fig. 1a, where an increase in TPC was achieved at lower amplitudes and shorter extraction times. In blackberry residues a different trend was reported where quadratic amplitude and time were significantly at $p < 0.0001$ (Zafra-Rojas et al., 2016).

Ascorbic acid (AA) values ranged from 5366.8 to 6040.2 mg AAE/100 g dw (Table 2). The interaction between variables (β_{12}) had a significant effect ($p < 0.001$) on ascorbic acid content. Fig. 1b shows that longer extraction times and higher amplitudes increased the content of ascorbic acid in ultrasound samples.

Ultrasound has been reported to favor a rapid equilibrium during the dissolution of target compounds between plant cell walls and the extraction solvents (Zou et al., 2014). However, long exposure to ultrasound could degrade some components which require adjustments of the extraction conditions (Zhang et al., 2015) as temperature, which is critical when heat-sensitive substances such as phenolic compounds that are extracted because they degrade at high temperatures (Zou et al., 2011; Kuo et al., 2014; Wang et al., 2011). Therefore, in this study, the outlet temperature was controlled (40 ± 2 °C) for all samples.

DPPH• and ABTS•+ antiradical activity

Antioxidant activity measured by the DPPH• and ABTS•+ assays ranged from 49825 to 66365 µmol TE/100 g dw and 65437.5 to 87833.33 µmol TE/100 g dw, respectively (Table 2). The amplitude-time interaction (β_{12}) had a significant negative effect ($p < 0.0001$, Table 3) on the antioxidant activity measured by DPPH•, as shown in Fig. 2a this parameter decreased at shorter processing times and lower amplitudes. In contrast, the quadratic parameter of amplitude (β_{11}) had a positive significant effect on the antioxidant activity by ABTS•+ ($p < 0.0001$, Table 3 and Fig. 2b). Similar behavior was reported by Zafra et al., (2016) in DPPH•, while ABTS was the quadratic of time.

Optimization of the ultrasound extraction conditions

Fig. 3 shows the optimization obtained by overlapping of the contour plots. The best results for TPC, AA, DPPH• and ABTS•+ antiradical activity together with the shorter processing time were the criteria selected to determine the optimal processing conditions. Ultrasound applied at amplitude of 91% for 7 min reaching 8800 mg GAE/100 g dw and 5600 mg AAE/100 g dw of TPC and AA, respectively, while antiradical activity by DPPH• and ABTS•+ were of 60000 µmol TE/100 g dw and 75000 µmol TE/100 g dw, respectively. To confirm these results, triplicate assays were performed under these optimized conditions and compared with conventional extraction methods.

Reproducibility of the study

Comparison between experimental (optimal condition process) and predicted values was performed and the experimental and predicted values are shown in table 4 where TPC and AA were similar ($p \geq 0.05$), while the antioxidant activity by DPPH• and ABTS•+ showed significant differences. These indicate that only the TPC and AA parameters are reproducible applying the ultrasound optimal process condition.

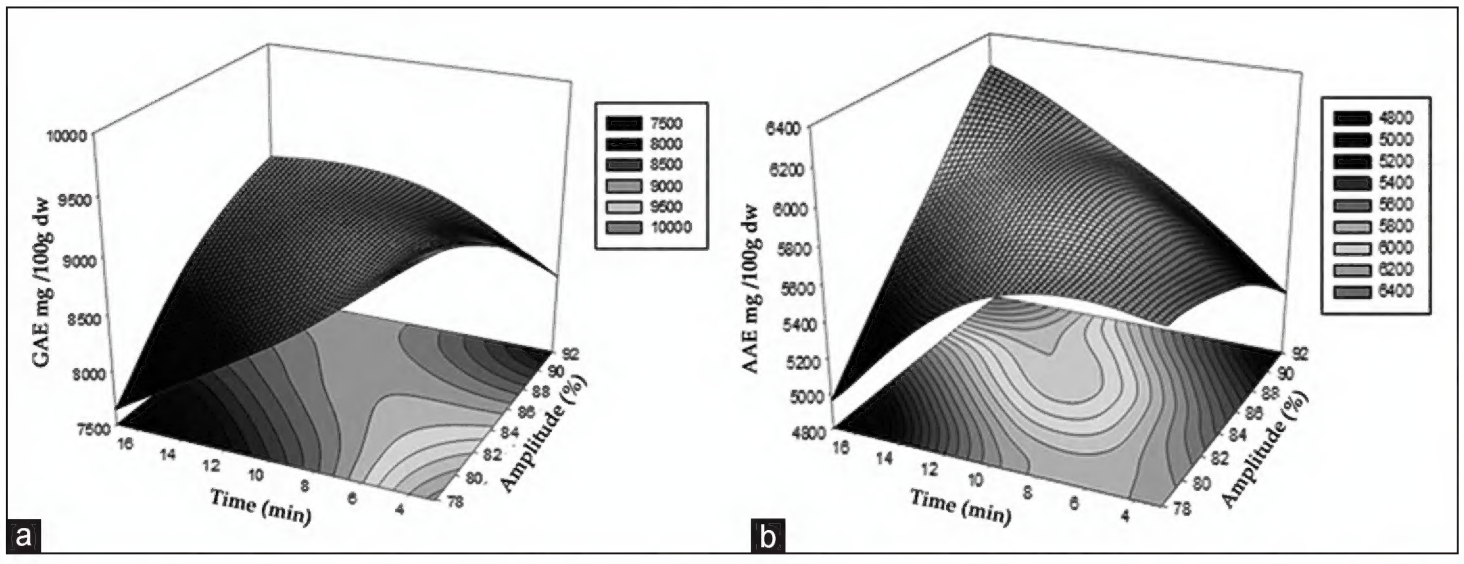

Fig 1. Response surface plots for antioxidant content relative to ultrasound amplitude (β_1) and extraction time (β_2). (a) Total phenolic content (mg GAE/100 g dw); and (b) Ascorbic acid (mg AAE/100 g dw) in mango waste subjected to ultrasound extraction.

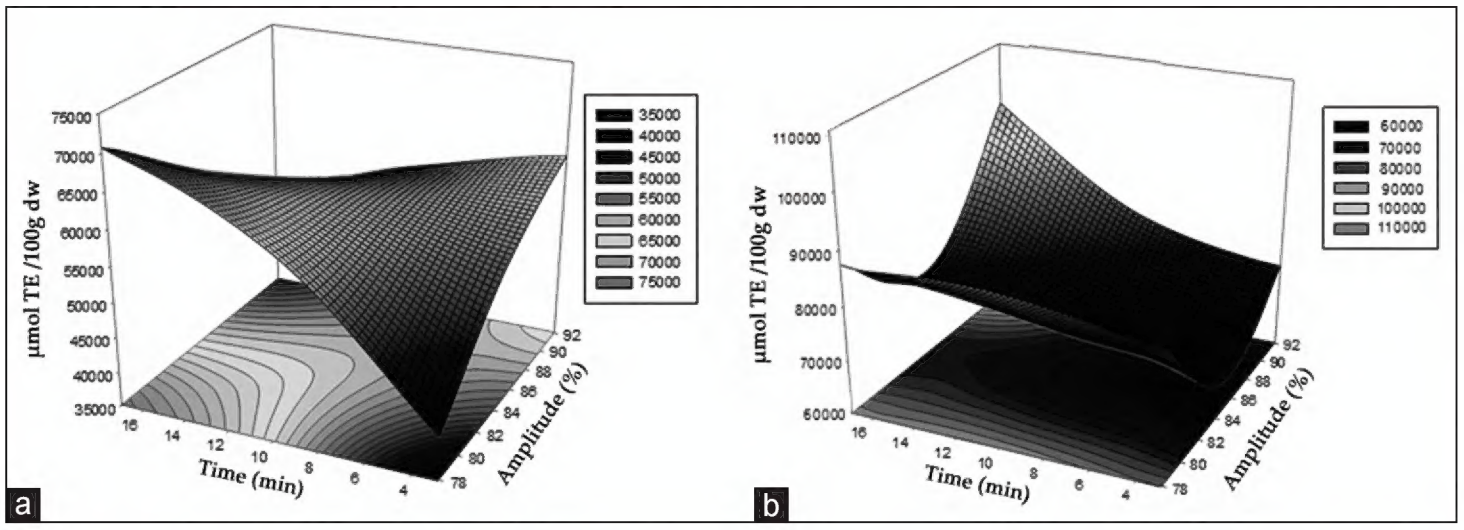

Fig 2. Response surface plots for antioxidant activity relative to ultrasound amplitude (β_1) and extraction time (β_2). (a) DPPH•; (b) ABTS•⁺, (µmol TE 100 g^{-1} dw).

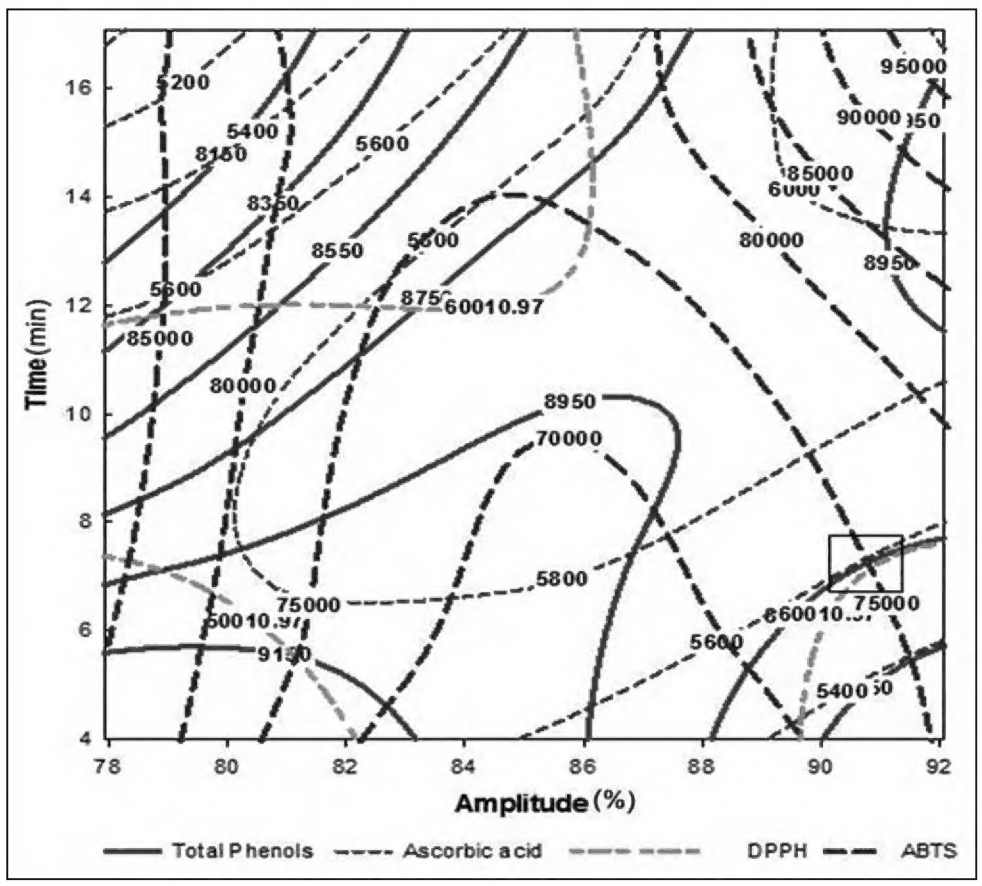

Fig 3. Overlapping contour plot. The optimal region in the area inside the box (□) corresponded to ultrasound processing conditions of 91% and a processing time of 7 minutes.

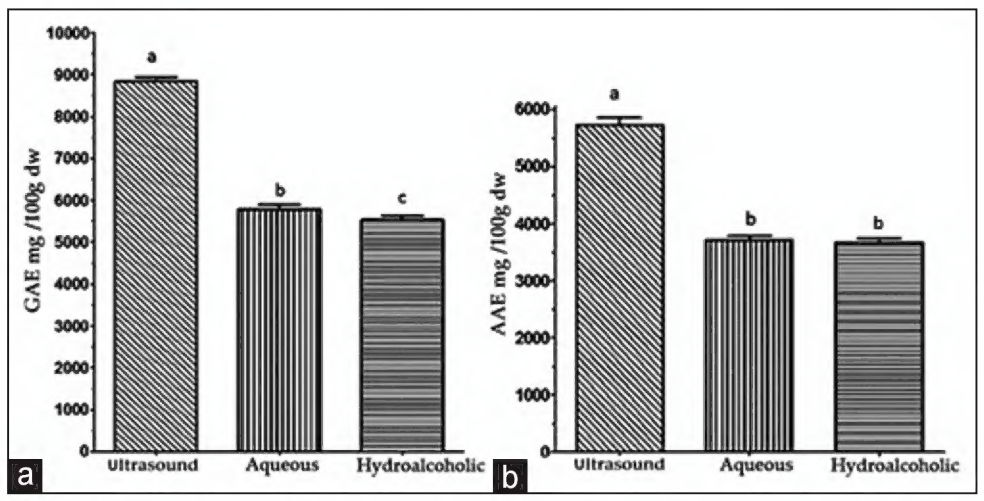

Fig 4. Comparison of antioxidant content extracted by different methods (a) Total phenolic content (mg gallic acid equivalent 100 g dry weight basis), and (b) Ascorbic acid (mg ascorbic acid equivalent 100 g dry weight basis). [a-c]Different letters indicate significant differences (p<0.05) between samples.

Table 4: Predicted and experimental values at the optimal ultrasound conditions

Parameters	Predict values	Experimental values
TPC (mg GAE/100 g dw)	8800	8837.68±105.9
Ascorbic acid (mg AAE/100 g dw)	5600	5730.67±127.8
DPPH• (µmol TE/100 g)	60000	60080±379.0*
ABTS•⁺ (µmol TE/100 g)	75000	76618.1±1752.4*

*Indicate significant differences (p<0.05) between predicted and experimental values according to the *t*-test

Fig. 4 shows the results for TPC and AA extracted by conventional methods and under optimized conditions. The highest values ($p \leq 0.05$) of TPC with 8837.68 mg GAE/100 g dw and for AA with 5730.67 mg AAE/100 g dw (Fig. 4a and Fig. 4b, respectively) were achieved by the optimized ultrasound compared with conventional methods which presented values of TPC of

5783.34 mg GAE/100 g dw and 5223.16 mg GAE/100g dw to aqueous and hydroalcoholic, respectively, while in AA conventional methods no presented significant different with mean of 3700.15. Similar results in TPC were observed by Zafra et al., (2016), although with values lowest.

With respect to antiradical activity by DPPH• and ABTS•$^{+}$, the optimized ultrasound extraction method compared with conventional methods had significantly higher values exhibiting 60080 µmol TE/100 g dw and 76618 µmol TE/100 g dw, respectively (Fig. 5a and b), conventional methods presented similar values ($p \geq 0.05$) in DPPH• (30750 µmol/TE 100 g dw), while ABTS•$^{+}$ the aqueous extraction had 40534.10 µmol TE/100 g dw and the hydroalcoholic 40800.54 µmol/TE 100 g dw. The behavior of ultrasound extraction was similar to Zafra et al., (2016) which was high in comparison with conventional methods. The increase of antioxidant compounds is due to the amplitude increase that had an effect on cell walls allowing its release (Patist and Bates, 2008), also the ultrasound could cause partial or total breaking of matrix allowing that compounds are available in the solvent (Hossain et al., 2012) in as much as sonication provide hydration and fragmentation process achieving the mass transfer of compounds to solvent (Toma et al., 2001).

These results demonstrated that the selected model was adequate to find the optimal ultrasound amplitude percentage and extraction time for processing mango waste. According to reported by Sumaya-Martinez (2019) the bioactive compounds of by-products obtained in mango are comparable with different types of cereals. In addition, other studies showed antioxidant activities important on seed and peel (by-products of mango) (Choudhary et al., 2023; García-Mahecha, et al., 2023; Lebaka et al., 2021; Sumaya-Martinez et al., 2019). The ultrasound extraction application in the by-product of mango in the present study, allowed the release of compounds obtaining high values of TPC and antioxidant activity. Therefore, the by-products of the mango fruit (peel, seed and pulp adhered to both) could be an ingredient with high added value for the food industry as an additive and as an ingredient in functional foods with the use of ultrasound.

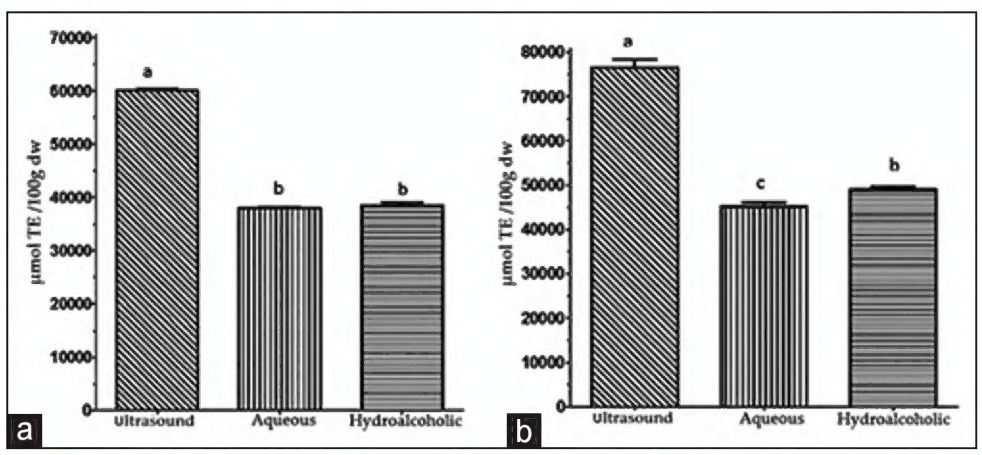

Fig 5. Comparison of antioxidant activity obtained by different extraction methods (a) DPPH• (µmol Trolox equivalent 100 g dry weight basis), (b) ABTS•$^{+}$ (µmol Trolox equivalent 100 g dry weight basis). $^{a\text{-}c}$ Different letters indicate significant differences ($p<0.05$) between samples.

CONCLUSION

Ultrasound technology could be a valuable tool for mango agribusiness to generate an added value product from waste and reduce pollution. The ultrasound conditions at amplitude of 91 % for 7 min extraction proved to be a suitable option to efficiently extract antioxidant compounds from mango waste in comparison to conventional methods. Further research is required to determine the potential health benefits of the extracted antioxidant compounds from this waste. Our findings may contribute to research on this regard.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

Author´s contributions

Raquel Cariño Cortés, Nelly del Socorro Cruz-Cansino, Esther Ramírez-Moreno, Eli Mireya Sandoval-Gallegos designed the experiments and analyzed the data, in addition to be directors of thesis of Raymundo Neria-de la Cruz, performed the experiments; María Teresa Sumaya-Martínez and Eduardo Fernández-Martínez contributed to valuable discussion and contributed with the with reagents/materials/analysis tools.

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